DETAILED ACTION

Status of the Application

1. Receipt is acknowledged of a response dated on March 21, 2008.

Status of the Claims

2. Claims 1, 3-70, 72, 75-77 and 138-142 were pending. Applicants canceled claims 1, 3-68, 75, 138 and 139. In addition, claims 140 and 141 were amended. Therefore, claims 69, 70, 72, 76, 77, and 140-142 are currently pending and examined on the merits.

Withdrawn Rejections/Objections

3. All outstanding rejections/objections are withdrawn in view of Applicants' amendments/cancellation of claims.

Reasons for Allowance

4. The claims are allowed because the prior art of record neither teaches nor fairly suggests Applicants' claimed screening methods. For example, Swanson et al. (e.g., see 11/16/07 Final rejection, page 6, 102(b) rejection against canceled claim 75) fail to teach the currently claimed quencher-linker-fluorophore chemical moiety as recited in claim 69. Swanson et al. only teach a quencher-fluorophore (e.g., see figure 4 wherein the ZFR-CMAC is disclosed). Furthermore, the ZFR-CMAC moiety could not have been modified (or viewed as) a quencher-linker-fluorophore because the bond between the ZFR quencher and the CMAC fluorophore is what controls fluorescence. For example, if an additional "carbonyl linker" were to be added to make a ZFR-(C=O)-CMAC molecule (instead of ZFR-CMAC) then the linker (i.e., the additional C=O unit),

not the ZFR, would quench the CMAC molecule upon formation of the bridging covalent linkage. Please note that "quenching" has been defined broadly in Applicants' specification to include any process that reduces the fluorescence intensity of the fluorophore and is not just limited to FRET (see specification, "Internally Quenched Reporters" section, "Some quenchers absorb fluorescence emitted from the fluorophore (e.g., via fluorescence energy transfer (FRET) mechanisms). Other quenchers modify the electronic properties of the fluorophore so as to interfere with the ability of the fluorophore to emit fluorescence"). In effect, the C=O group (or perhaps the ZFR-(C=O) group in its entirety) would become the "new" quencher rather than the original ZFR in this hypothetical. That is, the ZFR-(C=O)-CMAC would still represent a quencher-fluorophore rather than the claimed quencher-linker-fluorophore because the C=O is the chemical moiety that interferes with (i.e., quenches) the electronic properties of the CMAC molecule. This is so because the lone pair of electrons on the free nitrogen atom of the CMAC molecule (i.e., the free amino group) is what controls the fluorescence of the CMAC molecule (see Swanson et al., figure 4 showing free amino group on cleaved CMAC inside vacuole). When the lone pair of electrons is available for delocalization into the CMAC ring system as is the case for the cleaved CMAC molecule (see figure 4) then fluorescence can occur. When the lone pair of electrons is "tied up" by the formation of a CO-NH bond (as is the case for ZFR-CMAC molecule wherein the arginine (R) donates the CO portion and the CMAC donates the NH portion of the amide linkage) or, alternatively, the CO linker donates the CO in the above ZFR-CO-CMAC hypothetical, the electrons are no longer in a position to delocalize into the ring system and quenching results. Thus, any chemical functionality (including a "linker") that traps this lone pair of electrons (alters its electronic properties) will function as a quencher. In

addition, any linker that prevents the CMAC from forming an amide bond but does not "tie up" the lone pair of electrons on the free amino group of the CMAC molecule (as did the above C=O group) will negate quenching all together. That is, neither the linker nor the ZFR will be able to function as a quencher. For example, a linker like $-CH_2-$ will not "tie up" the lone pair of electrons on the CMAC amino group in the same manner as did the (C=O) group mentioned above because the $-CH_2-$ grou;p does not contain an "electron sink" (i.e., an electronegative carbonyl group). Further, this $-CH_2-$ will prevent the electronegative (C=O) group on the ZFR from quenching the CMAC because it will now be separated from the CMAC by one methylene unit (i.e., no longer conjugated to the nitrogen). That is, the ZFR portion of a compound like ZFR-CH_2-CMAC will not quench the CMAC group at all because it will be "blocked" from doing so by the linking $-CH_2-$ group. Thus, any linker that binds to the CMAC group will either act as a quencher as set forth in the first ZFR-CO-CMAC hypothetical or, alternatively, prevent the ZFR group from quenching the CMAC (i.e., neither the linker nor the ZFR will act as a quencher) as set forth in the second ZFR-CH_2-CMAC hypothetical. In either case, the claimed limitation "the fluorophore is linked to the quencher by a linker susceptible to cleavage within the cell" will not have been met.

In addition, even if, *assuming arguendo*, it could be stated that the ZFR could still function as a quencher with a linker in between (the linker in this scenario not acting as a quencher itself) there would still be no motivation to make this change. The addition of a linker would likely jeopardize the ability of the proteolytic enzyme to cleave the substrate (i.e., ZFR-linker-CMAC would likely be a worse substrate than ZFR-CMAC for the proteolytic enzymes). Enzymes like the proteases disclosed in Swanson et al. generally require very specific enzyme-

substrate interactions (e.g., for the stabilization of a transition state). And any changes in this regime will usually destroy the binding affinity and/or catalytic activity of the enzyme. In addition, even if, *assuming arguendo*, a ZFR-linker-CMAC molecule could act as a substrate for the enzyme (even though there is no evidence of record to suggest this), no benefit would be obtained. The CMAC is already quenched by the ZFR and the addition of a linker would not further quench the molecule. Likewise, cleavage of the CMAC from a ZFR-linker (as opposed to just the ZFR) would not further increase the fluorescence intensity of the cleaved CMAC. Finally, substituting an entirely new quencher-linker-fluorophore for the ZFR-CMAC moiety would not work either as the CH_2Cl group on the ZFR-CMAC is required for transport (see figure 4, $CH_2Cl \rightarrow CH_2SG$ transition). That is, it's unclear whether any other quencher-linker-fluorophore would be transported at all and, if so, whether a library would be generated like the CH_2Cl and CH_2SG molecules disclosed by Swanson et al. during the course of that metabolic processing (see Swanson et al., figure 4).

With regard to the other claims, Swanson et al. also fail to teach the use of different reporters as recited in claim 76. The only library members containing the claimed compound-reporter structures recited in Swanson et al. are the R-CH_2Cl and R-CH_2SG compounds noted above, which are both labeled with the same CMAC reporter. Furthermore, there would be no motivation to change this reporter because Swanson et al. is not interested in identifying various components of the metabolic pathway but, rather, using a label like ZFR-CMAC (regardless of how it's processed) to distinguish different types of cellular organelles. Blevitt et al. also fail to teach the use of "preparing a modified complex … identified in step (c) and a pharmaceutical agent" as set forth in independent claim 77. Finally, Swanson et al. also fail to disclose the use

of reporters that bind to nucleic acids, promote aggregation, and transcription (as in independent claims 140-142) and it is unclear whether any of these types of labels known in the art could be modified to function inside the vacuoles as disclosed by Swanson et al. to achieve the same effect.

Although Blevitt et al. (see 11/8/04 IDS, reference C1) was not discussed in any previous rejection (e.g., see 11/8/04 IDS, reference C1), it is also considered relevant prior art. Blevitt et al. disclose a method for screening carrier-mediated transport proteins (i.e., TAP) against a library of 136,928 compounds wherein a "compound-reporter" was used to monitor the activity (e.g., see Blevitt et al., abstract; see also figure 1 wherein FITC-RYNATRGL and RITC-RYNATROL are disclosed). However, Blevitt et al. fail to teach the use of a reporter that "preferentially generates the signal once internalized within the cell" as recited in claim 69. The FITC and RITC labels generate signals regardless of whether they are in the cell or not. In addition, Blevitt et al. fail to teach providing a library comprising different complexes, each complex comprising a compound and a separate reporter, the compound varying between different complexes and contacting one or more cells with a plurality of complexes from the library simultaneously as recited in independent claims 69, 76, 77, and 140-142. The 136,928 compounds disclosed in Blevitt et al. are not attached to any fluorophore and thus do not qualify as complexes within the meaning of the claims. Blevitt was using these compounds solely to study their inhibitory effects against known transport proteins that were fluorescently labeled for the purposes of high throughput screening (i.e., a high throughput screening inhibition assay using FITC-RYNATRGL). Thus, a person of skill in the art would not have been motivated to label each library member because the inhibition in this study was measured indirectly through

the use of a known, labeled peptide rather than through the measurement of each library member directly. Labeling 136,928 molecules separately would result in a time consuming, expensive process that may not work because the effects of each label on each substrate might alter the "true properties" of the substrate just as the FITC/RITC altered the transport properties of the RYNATRGL substrate. In addition, the FITC/RITC-RYNATRGL could not be considered as the library either since (1) these two molecules were tested separately, not simultaneously, and (2) these two molecules did not contain a "different compound" but, rather, the same RYNATRGL compound. Further, Blevitt was merely trying to characterize the FITC/RITC for future use in the inhibition assay, not test/produce a library of different reporters each bearing a different compound. Blevitt also fail to teach the step of "preparing a modified complex … comprising the compound identified in step (c) and a pharmaceutical agent" as in independent claim 77. Likewise, for independent claims 140, 141, and 142, Blevitt et al. also fail to teach the use of reporters that bind nucleic acid, promote aggregation or promote transcription. And it is unclear whether an assay system employing these types of reporters could be developed using TAP proteins and whether they would provide any benefit over the assay developed by Blevitt.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Salutation

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jon D Epperson whose telephone number is (571) 272-0808. The examiner can normally be reached Monday-Friday from 9:00 to 5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James (Doug) Schultz can be reached on (571) 272-0763. The fax phone number for the organization where this application or proceeding is assigned is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571) 272-1600.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Jon D. Epperson/
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